Tumor Necrosis Factors: Gene Structure and Biological Activities

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Tumor necrosis factor (TNF) is the name given to a serum-derived factor that is cytotoxic for many transformed cell lines in vitro and causes the necrosis of certain tumors in vivo (Carswell et al. 1975). The name lymphotoxin was proposed in 1968 for a factor with similar biological properties that is synthesized by mitogen-stimulated lymphocytes (Granger and Kolb 1968; Ruddle and Waksman 1968). Both of these activities are now known to correspond to distinct proteins. Many other proteins with cytotoxic activities have been described and given a variety of names; however, whether any of these activities can be attributed to cytokines distinct from TNF and lymphotoxin is still not clear. On the basis of structural homology and similarity in biological function, the names TNF-α and TNF-β have been given to TNF and lymphotoxin, respectively. Reviews on the current status of research on TNF-α (Old 1985; Pennica and Goeddel 1986) and TNF-β (Gray 1986) have appeared recently.

TNF Protein and cDNA Structure

Both TNF-α and TNF-β are assayed by measuring cytotoxic activity on actinomycin-D-treated L-M cells, a sensitive clone of murine L929 fibroblasts (Kramer and Carver 1986). The biochemical characterization of the TNFs was greatly aided by the identification of cell lines capable of producing these cytokines after exposure to phorbol myristate acetate (PMA). TNF-α and TNF-β were found to be produced by the human promyelocytic cell line HL-60 (Pennica et al. 1984) and the human lymphoblastoid cell line RPMI 1788 (Aggarwal et al. 1984), respectively. Both proteins were purified to homogeneity by Aggarwal et al. (1984, 1985b,c). Amino acid sequence analysis of the purified cytokines permitted the design of synthetic DNA hybridization probes that were used to screen the appropriate cDNA libraries. Cloned cDNAs corresponding to human TNF-α (Pennica et al. 1984) and TNF-β (Gray et al. 1984) were isolated and characterized.

On the basis of sequence analysis of cloned cDNAs, human TNF-α mRNA was shown to encode a precursor protein of 233 amino acids (Pennica et al. 1984). Amino-terminal sequence analysis of natural TNF-α (Aggarwal et al. 1985c) demonstrated that the mature protein of 157 amino acids is preceded by a 76-amino-acid signal sequence involved in protein secretion. The calculated monomeric molecular weight of 17,356 agrees with the value determined experimentally by SDS-PAGE under reducing conditions. The two cysteine residues of the mature TNF-α are linked by a disulfide bridge that is essential for cytotoxic activity (Aggarwal et al. 1985c).

TNF-β mRNA contains an open reading frame of 205 codons, the first 34 of which constitute a secretion signal sequence. Native TNF-β isolated from the RPMI 1788 cell line is a glycoprotein that exists in two forms (20 kD, 148 amino acids, and 25 kD, 171 amino acids) differing by 23 amino acids at their amino termini (Aggarwal et al. 1985b). Unlike TNF-α, TNF-β does not contain any cysteine residues.

A comparison of TNF-α and TNF-β reveals a high degree of amino acid sequence homology. If two gaps are introduced, the sequences can be aligned so that 44 amino acids (28%) occur in identical positions (Fig. 1). The introduction of two additional gaps permits the alignment of nine more amino acids (34% overall homology; Aggarwal et al. 1985c). It is likely that the two highly conserved regions (amino acids 35–66 and 110–133; TNF-α numbering) are important for the similar cytotoxic activities of the two molecules and/or the recognition of the TNF receptor. Interesting differences between the two proteins are found in the amino-terminal portion and in the region from amino acids 67 to 109 (TNF-α numbering), where there are only two identical residues.

Expression plasmids were constructed that direct the synthesis in *Escherichia coli* of mature recombinant human TNF-α (Pennica et al. 1984) and TNF-β (Gray et al. 1984). Both recombinant products have been purified to homogeneity free of contaminating lipopolysaccharide (LPS or endotoxin). Specific activities of approximately 10^8 U/mg were determined for both TNFs in the L-M cell in vitro cytotoxic assay.

The amino acid sequences of TNF-α (murine, rabbit, and bovine) and TNF-β (murine and bovine) from other species have been deduced by cDNA and genomic DNA sequencing. These amino acid sequences are compared in Figures 2 and 3. The high degree of amino acid con-

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TNF-α                                                                    1
TNF-β   leu pro gly val gly leu thr pro ser ala ala gln thr ala arg gln his pro lys met val arg ser
        1                                   10                                  20

                                10                                  20
TNF-α   ser ser arg thr pro ser asp lys pro val ala his val val ala asn pro gln ala glu
                                  *   *       *   *                 *
TNF-β   his leu ala his ser thr leu lys pro ala ala his leu ile gly asp pro ser lys gln
                                    30                                  40

                                30                                  40
TNF-α   gly gln leu gln trp leu asn arg arg ala asn ala leu leu ala asn gly val glu leu
                  *       *                     *       *           *           *
TNF-β   asn ser leu leu trp arg ala asn thr asp arg ala phe leu gln asp gly phe ser leu
                                    50                                  60

                                50                                  60
TNF-α   arg asp asn gln leu val val pro ser glu gly leu tyr leu ile tyr ser gln val leu
                  *       *     *   *           *       *           *   *   *   *
TNF-β   ser asn asn ser leu leu val pro thr ser gly ile tyr phe val tyr ser gln val val
                                    70                                  80

                                70                                  80
TNF-α   phe lys gly gln gly cys pro ser thr his val leu leu thr his thr ile ser arg ile
          *       *
TNF-β   phe ser gly lys ala tyr ser pro lys ala thr ser ser pro leu tyr leu ala his glu
                                    90                                  100

                                90                                  100
TNF-α   ala val ser tyr gln thr lys val asn leu leu ser ala ile lys ser pro cys gln arg
                                                          *
TNF-β   val gln leu phe ser ser gln tyr pro phe his val pro leu leu ser ser gln lys met
                                   110                                 120

                               110                                  120
TNF-α   glu thr pro glu gly ala glu ala lys pro trp tyr glu pro ile tyr leu gly gly val
              *           *           *   *                       *       *
TNF-β   val tyr pro gly leu gln glu --- --- pro trp leu his ser met tyr his gly ala ala
                                   130

                               130                                  140
TNF-α   phe gln leu glu lys gly asp arg leu ser ala glu ile asn arg pro asp tyr leu asp
          *   *   *           *   *       *   *                                   *
TNF-β   phe gln leu thr gln gly asp gln leu ser thr his thr asp gly ile pro his leu val
        140                                 150

                               150
TNF-α   phe ala glu ser gly gln val tyr phe gly ile ile ala leu
                  *           *       *   *               *   *
TNF-β   leu ser pro ser thr --- val phe phe gly ala phe ala leu
        160                                             170
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Figure 1. Comparison of the amino acid sequences of TNF-α and TNF-β. Two gaps have been introduced into the TNF-β sequence to increase the homology. Asterisks indicate identical amino acids.

servation among the various TNF-α and TNF-β sequences is not surprising in view of the cross-species activities observed for these proteins (Pennica et al. 1985).

TNF Gene Structure

TNF-α and TNF-β are homologous cytokines that share many biological properties, yet are produced by distinct cell types. To understand more precisely their differential regulation, we compared the structure and organization of the human TNF genes (Nedwin et al. 1985). Human TNF-α and TNF-β were found to each be encoded by a single gene. Furthermore, both genes are about 3 kbp in size and are split by three introns. However, only the third intron is located in a homologous position in the two genes (preceding amino acids 18 and 35 of TNF-α and TNF-β, respectively; see Fig. 1). Only the fourth exons, which encode the majority

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Human      -76  MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQR
Bovine     -77  MSTKSMIRDVELAEEVLSEKAGGPQGSRSCLCLSLFSFLLVAGATTLFCLLHFGVIGPQR
Rabbit     -79  MSTESMIRDVELAEGPLPKKAGGPQGSKRCLCLSLFSFLLVAGATTLFCLLHFRVIGPQE
Mouse      -79  MSTESMIRDVELAEEALPQKMGGFQNSRRCLCLSLFSFLLVAGATTLFCLLNFGVIGPQR
Consensus       MST SMIRDVELAE  L  K GG Q S  CL LSLFSFL VAGATTLFCLL F VIGPQ

                                        1
Human      -16  EEF-PRDLSLISPLAQA--VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGV
Bovine     -17  EEQVPSGPSINSPLVQ--TLRSSSQASSNKPVAHVVADINSPGQLRWWDSYANALMANGV
Rabbit     -19  EEQSPNNLHLVNPVAQMVTLRSASRALSDKPLAHVVANPQVEGQLQWLSQRANALLANGM
Mouse      -19  DEKFPNGLPLISSMAQTLTLRSSSQNSSDKPVAHVVANHQVEEQLEWLSQRANALLANGM
Consensus       E  P          Q    RS S   S KP AHVVA      QL W    ANAL ANG

Human       42  ELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPC
Bovine      42  KLEDNQLVVPAEGLYLIYSQVLFRGQGCP-PPPVLTHTISRIAVSYQTKVNILSAIKSPC
Rabbit      42  KLTDNQLVVPADGLYLIYSQVLFSGQGCR-SYVLLTHTVSRFAVSYPNKVNLLSAIKSPC
Mouse       42  DLKDNQLVVPADGLYLVYSQVLFKGQGCP-DYVLLTHTVSRFAISYQEKVNLLSAVKSPC
Consensus       L DNQLVVP  GLYL YSQVLF GQGC     LTHT SR A SY  KVN LSA KSPC

Human      102  QRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL.   157
Bovine     101  HRETPEWAEAKPWYEPIYQGGVFQLEKGDRLSAEINLPDYLDYAESGQVYFGIIAL    156
Rabbit     101  HRETPEEAEPMAWYEPIYLGGVFQLEKGDRLSTEVNQPEYLDLAESGQVYFGIIAL    156
Mouse      101  PKDTPEGAELKPWYEPIYLGGVFQLEKGDQLSAEVNLPKYLDFAESGQVYFGVIAL    156
Consensus       TPE AE   WYEPIY GGVFQLEKGD LS E N P YLD AESGQVYPG IAL
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Figure 2. Amino acid sequences of human (Pennica et al. 1984), bovine (D. Goeddel et al., unpubl.), rabbit (Itoh and Wallace 1985), and murine (Pennica et al. 1985) TNF-α. (-79 to -1) Amino acids of the signal sequences. "Consensus" indicates residues that are identical in all four sequences.

of the mature proteins, have significant DNA sequence homology (56%).

The human TNF genes were localized to the p23→q12 region of chromosome 6 through Southern blot analysis of human-murine somatic-cell hybrids (Nedwin et al. 1985). We have recently shown that the two genes are very closely linked; the polyadenylation site of the TNF-β gene is separated from the transcription-initiation site of the TNF-α gene by only 1221 bp. Both have the same orientation with respect to the direction of transcription. The regions immediately flanking the two genes are extremely homologous in the human,

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                                        1
Human      -34  MTPPERLFLPRVCGTTLHLLLLGLLLVLLPGAQGLPGVGLTPSAAQTARQHPKMHLAHST
Bovine     -33  MTPPGRLYLLRVCSTPP-LLLLGLLLALPLEAQGLRGIGLTPSAAQPAHQQLPTPFTRGT
Mouse      -33  MTLLGRLHLLRVLGTPP-VFLLGLLLALPLGAQGLSGVRF--SAARTAHPLPQKHLTHGI
Consensus       MT   RL L RV  T     LLGLLL L   AQGL G     SAA  A

Human       27  LKPAAHLIGDPSKQNSLLWRANTDRAFLQDGFSLSNNSLLVPTSGIYFVYSQVVFSGKAY
Bovine      27  LKPAAHLVGDPSTQDSLRWRANTDRAFLRHGFSLSNNSLLVPTSGLYFVYSQVVFSGRGC
Mouse       25  LKPAAHLVGYPSKQNSLLWRASTDRAFLRHGFSLSNNSLLIPTSGLYFVYSQVVFSGESC
Consensus       LKPAAHL G PS Q SL WRA TDRAFL  GFSLSNNSLL PTSG YFVYSQVVFSG

Human       87  SPKATSSPLYLAHEVQLFSSQYPFHVPLLSSQKMVYPGLQEPWLHSMYHGAAFQLTQGDQ
Bovine      87  FPRATPTPLYLAHEVQLFSPQYPFHVPLLSAQKSVCPGPQGPWVRSVYQGAVFLLTRGDQ
Mouse       85  SPRAIPTPIYLAHEVQLFSSQYPFHVPLLSAQKSVYPGLQGPWVRSMYQGAVFLLSKGDQ
Consensus       P A   P YLAHEVQLFS QYPFHVPLLS QK V PG Q PW  S Y GA F L  GDQ

Human      147  LSTHTDGIPHLVLSPSTVFFGAFAL     171
Bovine     147  LSTHTDGISHLLLSPSSVFFGAFAL     171
Mouse      145  LSTHTDGISHLHFSPSSVFFGAFAL     169
Consensus       LSTHTDGI HL  SPS VFFGAFAL
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Figure 3. Amino acid sequences of human (Gray et al. 1984), bovine (D. Goeddel et al., unpubl.), and murine (P.W. Gray, unpubl.) TNF-β. (-34 to -1) Amino acids of the signal sequences. "Consensus" indicates the residues that are identical in all three sequences.

murine, and bovine genomes (D.V. Goeddel et al., unpubl.).

Regulation of TNF-α and TNF-β Synthesis

TNF-α and TNF-β are produced by activated macrophages (Carswell et al. 1975) and lymphocytes (Granger and Kolb 1968; Ruddle and Waksman 1968), respectively (Stone-Wolff et al. 1984). We have examined the inducibility of both TNF-α and TNF-β by mitogens on 30 different cell lines representing many cell types. As TNF-α and TNF-β behave similarly in the L-M cell cytotoxic assay, they were distinguished by neutralization of activity with the corresponding monoclonal antibodies. Northern blot analysis using TNF-α and TNF-β cDNA probes was also performed to determine which TNF was expressed.

Although normal T lymphocytes have been reported to be the cellular source of TNF-β (Ruddle et al. 1983), we have not detected any TNF activity after exposure to the mitogen PMA using two human T-cell lines (Molt-4 and Jurkat). However, all six B lymphoblastoid cell lines examined produced high levels of TNF-β. Similar results were seen by Williamson et al. (1983), but they did not distinguish TNF-α and TNF-β activities.

The majority of the human and murine macrophage cell lines tested (including HL-60, U937, PU5-1.8, RAW 264, P388D$_1$, J774, and WRI-7) produce TNF-α but not TNF-β when stimulated with mitogens. This result was confirmed by Northern blot hybridization with the TNF-α and TNF-β cDNA probes. Other cell types, such as normal endothelial cells, rat glial cells (C6), IL-3-dependent normal bone-marrow-derived mast cells, and a mast cell line (A1) are capable of producing TNF-α but not TNF-β. Most nonlymphoid cell lines (including HeLa, A549, T24, WI38, K562, ME-180, HT-29, MCF, SKCO-1, A-431, HT1080, 7860, NRK, Rat-1, and C127) do not produce TNFs in the presence or absence of the mitogen.

The expression of both TNF-α and TNF-β mRNAs is transient even in the presence of continuous mitogenic stimuli. TNF-α mRNA in murine macrophage PU5-1.8 cells is detectable 1 hour after induction with PMA, is highest at approximately 2–4 hours, and becomes undetectable by 12 hours. In contrast, TNF-β mRNA in the B lymphoblastoid cell line 1788 begins to increase at 4 hours, reaches its maximal level at 12–24 hours, and decreases to the basal level after 48 hours. Thus, induction of TNF-α is faster than induction of TNF-β.

The effect of physiological mediators or cytokines on the expression of TNF was also examined. Glucocorticoids, known to be immunosuppressive agents (Claman 1972; Vischer 1972), have been shown to inhibit Ia antigen expression (Wong et al. 1984) and lymphokine production (Arya et al. 1984; Culpepper and Lee 1985). We found that the glucocorticoid dexamethasone inhibited TNF-α mRNA expression in HL-60 cells (Fig. 4). Similar results were obtained by Beutler et al. (1986) using murine macrophages. Other glucocorticoids (prenisolone, hydrocortisone, and corticosterone), but not sex steroids (testosterone, estrogen, and progesterone), inhibited the production of TNF-α in macrophage cell lines (HL-60, U937, PU5-1.8) and TNF-β in B lymphoid cells (RPMI 1788, IM9).

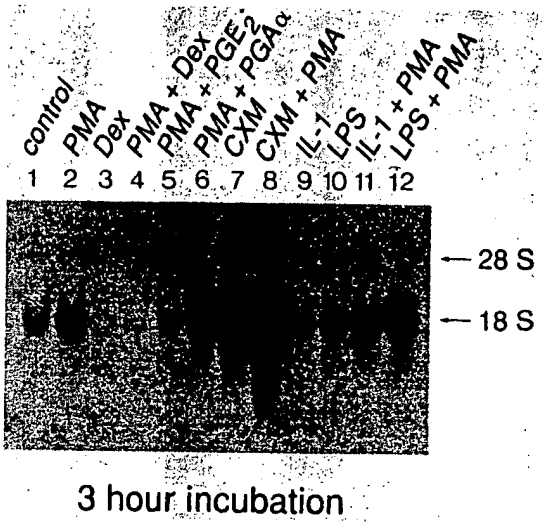

Figure 4. Regulation of TNF-α mRNA levels in HL-60 cells. HL-60 cells (5×10^6 cells/ml) were treated with PMA (50 ng/ml) in the presence or absence of dexamethasone (Dex) (5×10^{-7} M), PGE_2 (50 ng/ml), PGAα (50 ng/ml), cycloheximide (1 μg/ml), interleukin-1 (100 U/ml), and LPS (10 μg/ml). After 3 hr, total RNA was extracted, poly(A) RNA was prepared, and Northern hybridization was performed as described previously (Thomas 1980). Each lane contained about 1 μg of poly(A) RNA. Hybridization was performed using a ^{32}P-labeled TNF-α cDNA probe.

Prostaglandin E (PGE), which is an important modulator of inflammation and cellular immune responses (Goodwin and Webb 1980), also down-regulates the expression of TNF-α mRNA in HL-60 cells (Fig. 4), whereas prostaglandin Aα or Fα had no inhibitory effect. Similar inhibitory effects of PGE_1 and PGE_2 on the expression of TNF-α by other macrophage cell lines (U937, PU5-1.8, RAW 264, P388D$_1$, and J774) were observed. Indomethacin, a cyclooxygenase inhibitor (Sheen and Winter 1977), enhances the production of TNFs in both macrophages and B lymphoid cells. Surprisingly, PGE_1 or PGE_2 does not suppress the production of TNF-β mRNA or biological activity in two B lymphoid cell lines (RPMI 1788 and IM9). It will be of interest to examine how the differential expression of TNF-α and TNF-β is regulated by PGEs.

The protein synthesis inhibitor cycloheximide further enhances the accumulation of both TNF-α (Fig. 4) and TNF-β mRNAs induced by PMA. The production of TNFs by macrophages and lymphoid cells can also be enhanced by the lymphokine interferon-γ (IFN-γ).

Normally, LPS alone does not induce TNF-β in 1788 or IM9 cells. However, LPS in combination with PMA gave at least a tenfold greater increase in both TNF-β mRNA and biological activity than did PMA alone. Similarly, the induction of TNF-α mRNA in HL-60 cells by the combination is greater than by either in-

duction alone (Fig. 4). These results demonstrate that two different mitogens can synergistically induce the expression of both TNF-α and TNF-β genes. The regulation of the TNF genes by glucocorticoids, prostaglandins, cycloheximide, IFN-γ, and different mitogens observed in cell lines was similarly observed in normal human peripheral blood leukocytes. In addition to bacterial LPS and the mitogen PMA, both viruses and poly(I):poly(C) can induce the synthesis of TNF-α and TNF-β in TNF-producing cell lines and normal human leukocytes (Aderka et al. 1986; G. Wong and D. Goeddel, in prep.).

Antitumor Properties of TNFs

TNF is a term that was initially used to describe an activity present in the serum of bacillus Calmette-Guerin-infected endotoxin-treated mice that induced the hemorrhagic necrosis of certain transplantable tumors in inbred mice (Carswell et al. 1975). We have utilized this classic assay to compare further the ability of TNF-α and TNF-β to induce necrosis of an established Meth-A sarcoma intradermal implant in (BALB/c$\times$C57BL/6)F_1 mice. Intravenous injection of 1–50 μg of either recombinant human TNF-α (r-hTNF-α) or r-hTNF-β induced significant degrees of hemorrhagic necrosis 24 hours after injection (Table 1). We have previously reported similar antitumor activity following intraperitoneal, intralesional, and intramuscular injections of TNF-α and TNF-β (Gray et al. 1984; Pennica et al. 1984).

Additional experiments were performed to characterize the in vivo antitumor activities of TNF-α against two subcutaneously implanted, chemically induced sarcomas of BALB/c origin (WEHI-164 and Meth A). Intravenous treatment with TNF-α significantly inhibited the growth of both sarcomas (Table 2). However, there were no complete remissions in the WEHI-164 group and only one of ten animals in the Meth-A group completely rejected the tumor after TNF-α treatment. The site of tumor implantation appears to be a critical factor for the demonstration of antitumor activities of TNF-α against transplantable sarcomas, since Meth A and M5076 (a spontaneously arising ovarian sarcoma of C57BL/6 origin) implanted intraperitoneally are almost completely refractory to the antitumor effects of TNF-α (M.A. Palladino, unpubl.). In contrast, treatment of the Meth-A tumor implanted intradermally results in a cure rate of almost 100%.

We have previously reported the ability of both TNF-α and TNF-β to augment superoxide radical production, antibody-dependent cellular cytotoxicity, and phagocytosis by neutrophils (Shalaby et al. 1985). The role of neutrophils in host defense responses is well established, and they have been shown to release activated oxygen intermediates (Babior 1978) and interleukin-1 (Tiku et al. 1986). Other studies have shown that neutrophils can mediate endothelial damage in vitro and that TNF-α can directly stimulate endothelial cells to produce procoagulant activity (Harlan et al. 1981; Nawroth and Stern 1986). It is therefore quite possible that the association between changes in neutrophil functions and endothelial cell homeostatic properties mediates the antitumor activities of TNF in vivo (Pennica et al. 1986). This hypothesis is supported by the fact that intraperitoneally implanted Meth A is refractory to the antitumor effects of TNF-α, whereas intradermally implanted Meth A is highly sensitive. Studies directed at addressing this hypothesis further are in progress and may give greater insight into the in vivo antitumor mechanisms of TNF.

In Vitro Growth Activities of TNFs

TNF-α and TNF-β have been shown to have comparable cytostatic and cytolytic properties in vitro (Ag-

Table 1. Necrosis of Meth-A Sarcoma after Intravenous Injection of r-hTNF-α or r-hTNF-β

Intravenous treatment	Dose (μg)	Hemorrhagic necrosis score				Percentage of mice with >25% necrosis
		+ + +	+ +	+	−	
PBS		0	0	2	18	0
r-hTNF-α	50	15	3	1	0	95
	15	13	6	1	0	95
	5	13	5	0	1	95
	1	3	7	7	3	50
r-hTNF-β	50	14	1	4	1	75
	15	15	2	2	1	85
	5	11	5	2	2	80
	1	4	6	6	4	50

(BALB/c × C57BL/6)F_1 female mice were injected intradermally with 5×10^5 Meth-A sarcoma cells. After 7 days (average tumor diameter 0.75 cm), TNF was injected intravenously in 0.1 ml PBS; 24 hr later, the tumors were excised, sectioned, and scored as described previously (Carswell et al. 1975; Pennica et al. 1984). + + + represents between 50% and 75% of tumor mass necrotic; + + represents between 25% and 50% of tumor mass necrotic; + represents less than 25% of tumor mass necrotic; − represents no visible necrosis.

Table 2. Antitumor Effects of r-hTNF-α against Subcutaneously Implanted Sarcomas

Sarcoma	Intravenous treatment	Tumor size (mm) on days 7	14	21	*p* Value
Meth A	PBS	2.6	7.1	15.1	
	r-hTNF-α	4.3	4.3	7.6	<0.01
WEHI-164	PBS	12.4	25.2	34.9	
	r-hTNF-α	9.3	14.3	22.9	<0.01

r-hTNF-α was administered daily from day 7 to day 18 for Meth A and from day 5 to day 12 for WEHI-164 at a dose of 25 μg/day and 15 μg/day, respectively. There were 10 animals per group.

garwal et al. 1984, 1985b,c). In addition, IFN-γ can potentiate the antiproliferative effects of both cytokines on certain tumor cell lines (Williams and Bellanti 1983; Williamson et al. 1983; Lee et al. 1984; Stone-Wolff et al. 1984; Sugarman et al. 1985). Subsequent experiments using recombinant TNF-α and TNF-β demonstrated that they compete for the same binding sites on tumor cells, which probably accounts for their similar effects on tumor cell growth in vitro (Aggarwal et al. 1985a).

A number of studies characterizing the effects of TNF-α or TNF-β on the proliferation of various cell lines have been reported (Williamson et al. 1983; Lee et al. 1984; Kull et al. 1985; Sugarman et al. 1985; Ruggiero et al. 1986). Initial results indicated that cell lines can be subdivided into three categories on the basis of their response to TNF-α: (1) a cytostatic or cytolytic effect, (2) little or no antiproliferative effect, and (3) enhanced growth (Table 3). Furthermore, addition of exogenous growth factors can either inhibit the antiproliferative response or enhance the growth-promoting effect of TNF-α (Vilcek et al. 1986; B.J. Sugarman et al., unpubl.). More recent results have shown that tumor cells sensitive to the cytotoxic effects of TNF-α are actually growth stimulated at relatively low concentrations of TNF-α (G. Lewis et al., unpubl.).

Approximately 40% of the established tumor cell lines are sensitive to the antiproliferative effects of TNF-α (Sugarman et al. 1985). Cells such as L929, WEHI 164, and UV1591-RE are extremely susceptible to TNF-α-mediated cytotoxicity (i.e., < 10 U/ml reduces cell viability by 50%), whereas a similar decrease in the viability of the most sensitive human cell lines (e.g., ME-180 and BT-20) requires a tenfold greater concentration of TNF-α. IFN-γ can enhance the cytotoxic effects of TNF-α on some TNF-sensitive cell types (e.g., ME-180 and BT-20) as well as cell lines that are insensitive to its antiproliferative effects (e.g., A549, B16F10, Saos-2, and WI38 VA13) (Sugarman et al. 1985, unpubl.). However, treatment of tumor cells with both IFN-γ and TNF-α does not always result in an enhanced cytotoxic response; no synergistic antiproliferative response is seen on T24 bladder carcinoma, Calu-3 lung carcinoma, or RPMI 7272 melanoma cell lines, which are all refractory to the cytotoxic effects of TNF-α alone (Sugarman et al. 1985; Tsujimoto et al. 1986).

The response of normal fibroblasts to treatment with TNF-α in vitro is completely different from that of other cell lines. Their growth is stimulated in the presence of picomolar concentrations of TNF-α (Sugarman et al. 1985; Vilcek et al. 1986). An antibody that neutralizes the cytotoxic effects of TNF-α on tumor cells abrogates TNF-α-induced proliferation of normal fibroblasts (Sugarman et al. 1985). IFN-γ interferes with TNF-α-induced growth of normal fibroblasts in a dose-dependent manner (Sugarman et al. 1985; Vilcek et al. 1986). This TNF-α-induced fibroblast proliferation can also be augmented by exogenously added factors. Insulin and platelet-derived growth factor enhance the proliferation of NRK-49F fibroblasts (G. Lewis et al., unpubl.).

It is not clear how TNF-α can stimulate the growth of certain cell lines while inhibiting the growth of others. Variations in the proliferative responses induced by TNF-α are not necessarily due to differences in the number of binding sites per cell or their affinity for TNF-α (Kull et al. 1985; Sugarman et al. 1985; Tsujimoto et al. 1986). The ability of TNFs to both stimulate and interfere with cell proliferation is similar to transforming growth factor-β, a distinct growth factor/inhibitor (Tucker et al. 1984; Roberts et al. 1985), and may be characteristic of proteins involved in homeostatic regulatory mechanisms.

Antiviral Properties of TNFs

In response to viral infection in vivo, interferons (IFNs) are secreted and induce a state of viral resistance in noninfected cells (Stewart 1981). TNF-α and TNF-β also have antiviral activity on some cells and antiviral enhancing activity on most cells tested.

TNF-α and TNF-β inhibit the cytopathic effects of vesicular stomatitis virus (VSV) in murine epithelial cells (C127) and rat fibroblasts (Rat-1). In addition to inhibition of the VSV-mediated cytopathic effect, TNF-α and TNF-β also dramatically decrease the VSV yield in these cells (Fig. 5). This antiviral activity is dose-dependent and is neutralized by respective monoclonal antibodies against human TNF-α and TNF-β. The antiviral activity of TNFs is not caused by the induction of IFNs from these cells, since the antiviral activity is not abolished by polyclonal or monoclonal antibodies against IFN-α, -β, or -γ. The antiviral effects of TNF-α and TNF-β are also observed with encephalomyocar-

Table 3. Response of Various Cell Lines to TNF-α or TNF-β In Vitro

Growth Enhancement	
CCD-18Co (normal human colon)	Sugarman et al. (1985)
Detroit 551 (normal human fetal skin)	Sugarman et al. (1985)
FS-4 (normal human foreskin)	Vilcek et al. (1986)
FS-48 (normal human foreskin)	Kohase et al. (1986)
LL24 (normal human lung)	Sugarman et al. (1985)
NRK-49F (normal rat kidney)	G.D. Lewis et al. (in prep.)
Osteoclasts	Bertolini et al. (1986)
WI38 (normal human fetal lung)	Lee et al. (1984); Sugarman et al. (1985)
WI-1003 (normal human lung)	Sugarman et al. (1985)
Null response	
A549 (human lung carcinoma)	Sugarman et al. (1985)
B16 (murine melanoma)	Lee et al. (1984)
B16F10 (murine melanoma)	Sugarman et al. (1985)
Calu-3 (human lung carcinoma)	Sugarman et al. (1985)
CMT-93 (murine rectal carcinoma)	Sugarman et al. (1985)
G-361 (human melanoma)	Sugarman et al. (1985)
HeLa (human cervical carcinoma)	Sugarman et al. (1985)
HeLa D98 (human cervical carcinoma)	Ruggiero et al. (1986)
HT-29 (human colon carcinoma)	Ruggiero et al. (1986); Tsujimoto et al. (1986)
HT1080 (human fibrosarcoma)	Sugarman et al. (1985)
KB (human oral epidermoid carcinoma)	Sugarman et al. (1985)
LS174T (human colon carcinoma)	Sugarman et al. (1985)
RD (human rhabdosarcoma)	Sugarman et al. (1985)
Saos-2 (human osteogenic sarcoma)	Sugarman et al. (1985)
SK-CO-1 (human colon carcinoma)	Sugarman et al. (1985)
SK-LU-1 (human lung carcinoma)	Sugarman et al. (1985)
SK-OV-3 (human ovarian carcinoma)	Sugarman et al. (1985)
SK-UT-1 (human uterine carcinoma)	Sugarman et al. (1985)
S49 (murine lymphoma)	Sugarman et al. (1985)
T24 (human bladder carcinoma)	Sugarman et al. (1985)
WI38 VA13 (human transformed WI38)	Lee et al. (1984); Sugarman et al. (1985)
Antiproliferative response	
BT-20 (human breast carcinoma)	Sugarman et al. (1985)
BT-475 (human breast carcinoma)	Sugarman et al. (1985)
B6MS2 (murine sarcoma)	Sugarman et al. (1985)
B6MS5 (murine sarcoma)	Sugarman et al. (1985)
CMS4 (murine sarcoma)	Sugarman et al. (1985)
CMS16 (murine sarcoma)	Sugarman et al. (1985)
L929 (murine fibroblast)	Sugarman et al. (1985)
MCF7 (human breast carcinoma)	Sugarman et al. (1985)
ME-180 (human cervical carcinoma)	Sugarman et al. (1985)
Meth A (murine sarcoma)	Sugarman et al. (1985)
MMT (murine breast carcinoma)	Sugarman et al. (1985)
SAC (Moloney-transformed murine 3T3)	Sugarman et al. (1985)
SK-MEL-109 (human melanoma)	Sugarman et al. (1985)
SK-OV-4 (human ovarian carcinoma)	Sugarman et al. (1985)
UV1591-RE (murine fibrosarcoma)	Urban et al. (1986)
WEHI-164 (murine sarcoma)	Sugarman et al. (1985)
WiDr (human colon carcinoma)	Sugarman et al. (1985)

ditis (EMCV) and herpes simplex virus types 1 and 2 (HSV-1 and HSV-2). Coincubation with the RNA synthesis inhibitor actinomycin D (1 μg/ml) or the protein synthesis inhibitor cycloheximide (1 μg/ml) abolishes the antiviral activity of TNFs. A 24-hour preincubation with TNF provides complete protection against VSV in C127 cells. The antiviral activity of TNF is also observed in human renal carcinoma 7860, lymphoid RPMI 8226, and murine macrophage RAW 264 cell lines. Human TNFs exhibit antiviral activity on some murine cell lines, and murine TNF-α also protects some human cells from viral infection. This suggests that the antiviral activity of TNF-α and TNF-β is not species-specific.

TNF-α or TNF-β alone protects against viral infection in some cell lines but is inactive in most cells. However, they enhance the antiviral activity of IFN-α, -β, and -γ on a variety of cell types tested. An example of their synergistic antiviral action using EMCV and the human lung carcinoma A549 cell line is shown in Figure 6. Similarly, the activity of bovine IFN-γ against VSV in bovine MDBK cells is also enhanced by human TNF-α or TNF-β.

Although IFN-γ is active in protecting most cells from VSV infection, 1 μg/ml (10^5 units in the EMCV assay) of human IFN-γ has no detectable activity against VSV in the A549 cell line. However, IFN-γ is effective in protecting against VSV infection in the

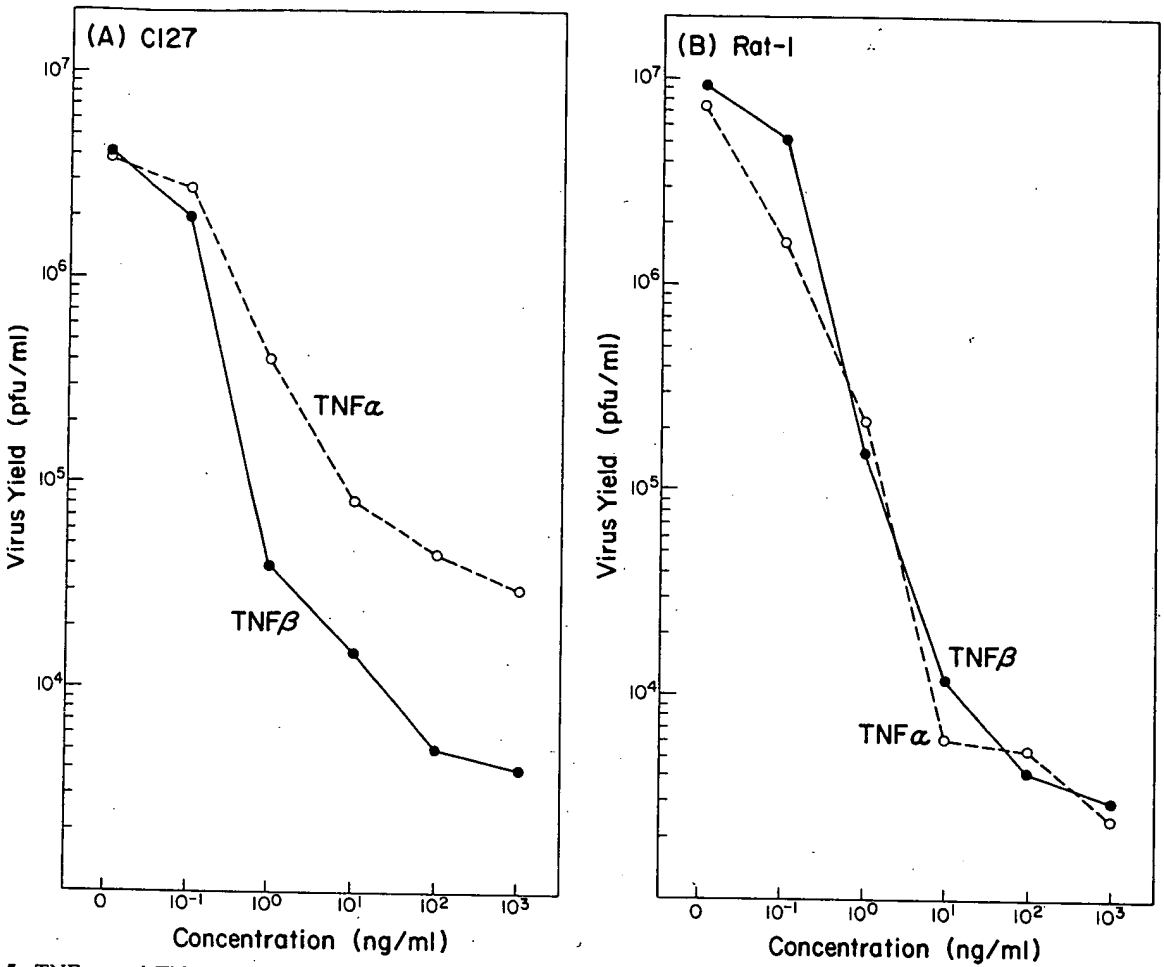

Figure 5. TNF-α and TNF-β inhibit VSV yield in murine C127 (*A*) and Rat-1 (*B*) cells. The cells were grown to confluency in 24-well plates and then treated wtih the indicated concentrations of TNF-α or TNF-β for 24 hr. The medium was removed before challenging with VSV at a multiplicity of infection (m.o.i.) of 10. Two hours later, the supernatants were aspirated to remove excess virus. After 24 hr, the cultures were assayed for virus yield in terms of plaque-forming units per milliliter on A549 cells (Rager-Zisman and Merigan 1973).

presence of TNF-α or TNF-β, although IFNs alone have no activity in these cells. As little as 1 ng/ml of IFN-γ and either TNF-α or TNF-β gave complete protection. Furthermore, this combination strongly inhibits the replication in A549 cells of the DNA viruses adenovirus-2, HSV-1, and HSV-2. IFN-γ alone is relatively ineffective. TNFs also enhance the antiviral activity of IFN-α and IFN-β, although to a lesser extent than IFN-γ.

TNF-α or TNF-β also enhances the antiviral activity of IFN-γ on a variety of transformed and normal cell lines. These include transformed cell lines of human (HeLa cervical carcinoma, HT1080 fibrosarcoma, 7860 renal carcinoma, T24 bladder carcinoma, HT-29 colon carcinoma, ST-486 Burkitt lymphoma, RPMI 8226 myeloma, and U87MG glioblastoma), rat (C6 glialoma), and murine (C127 epthelialoma and RAW 264 macrophage) origin, and three normal fibroblast cell lines (murine 3T3 and rat NRK and Rat-1). Thus, the antiviral potentiating activity of TNF-α or TNF-β is not virus-, cell-type-, or species-specific.

In addition to its preventive role against virus infection, TNF-α and TNF-β selectively kill virus-infected cells under certain conditions. This killing is enhanced by IFN-α, -β, or -γ. Thus, one major function of TNFs may be to broaden and extend the antiviral activity of IFNs by inducing cellular resistance in uninfected cells and by selectively destroying virus-infected cells. These results have implications for the therapy of the many medically important viral diseases caused by DNA and RNA viruses.

Effect of TNFs on HLA-B7 and HLA-DR Expression

The expression of the major histocompatibility complex (MHC) is essential for the initiation and regulation of the immune response. TNFs, like IFNs, can regulate the expression of both class I (HLA-B7) and class II (HLA-DR) MHC genes. Human bladder carcinoma T24 cells express detectable levels of HLA-B7 but not HLA-DRβ mRNA. Incubation with TNF-α (0.1 μg/ml) or IFN-γ (0.01 μg/ml) for 24 hours results in a fivefold increase of HLA-B7 but not HLA-DR mRNA. The induction is greater for the combination of IFN-γ and TNF-α than for either cytokine alone. Induction of HLA-B7 by IFN-γ is direct because an increase in

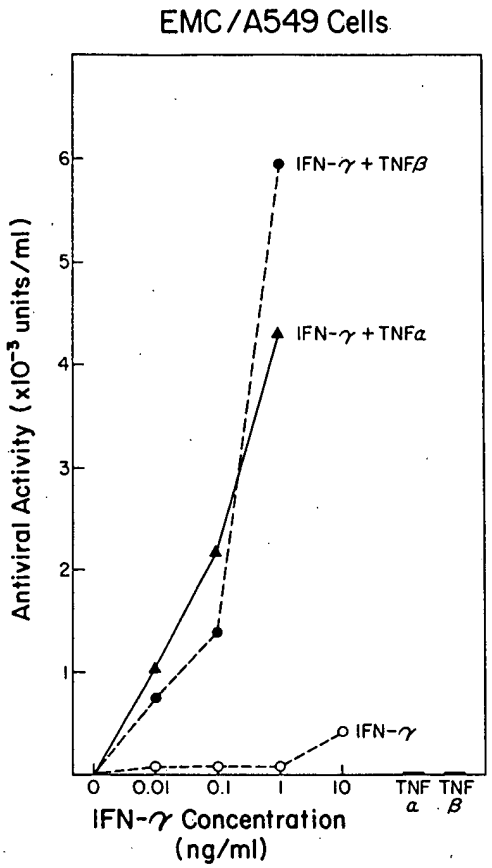

Figure 6. TNF-α and TNF-β enhance the antiviral activity of IFN-γ in A549 cells. A549 cells were grown to confluency, treated with human TNF-α (0.1 μg/ml), TNF-β (0.1 μg/ml), the indicated concentrations of IFN-γ, or the combinations for 24 hr before challenge with EMCV at a m.o.i. of 1. After 24 hr, the cytopathic effect was determined by staining the cells with crystal violet, and the titer was quantitatively monitored using a micro-ELISA autoreader. The assay was standardized to the International Reference Sample of the National Institutes of Health human IFN-γ (Gg 23-901-530).

HLA-B7 mRNA by IFN-γ can be detected after 4 hours of treatment in the presence of cycloheximide. In contrast, the induction of HLA-B7 by TNF-α can be blocked by cycloheximide, and the induction can only be detected after 12 hours of treatment. These results suggest that the induction of HLA-B7 mRNA by TNF-α is indirect. It is possible that TNF-α induces the synthesis of IFNs that cause the increase in HLA-B7 mRNA. However, the HLA-B7-inducing activity of TNF-α in T24 cells cannot be blocked by antibodies that neutralize IFN-α, -β, or -γ.

Although TNF-α and IFN-γ do not induce HLA-DR in most cell lines, they do induce HLA-DRβ mRNA in two human monocyte-like cell lines: the promyelocytic leukemia HL-60 and the histocytic lymphoma U937. The class-II-MHC-inducing activity of TNF-α is less potent than IFN-γ but is detectable at 24 hours. The combination of TNF-α and IFN-γ induces approximately tenfold higher levels of the HLA-DRβ mRNA in these cells than TNF-α or IFN-γ alone. TNF-β also enhances the expression of both HLA-B7 and HLA-DR mRNAs in HL-60 and U937 cells.

The expression of class I and class II MHC antigens has emerged as an essential component for antigen presentation in an immune response and in the control of tumor growth in vivo (Tanaka et al. 1985). Thus, TNFs and IFN-γ may potentiate the immune response through the synergistic amplification of MHC gene expression on a variety of cell types.

Catabolic Effects of TNFs

TNF-α is thought by some to be the agent that causes cachexia or wasting in parasite-infected animals (Beutler and Cerami 1986). However, there is no direct evidence to support this hypothesis. In cultured adipocytes, TNF can inhibit the transcription of genes encoding enzymes involved in fatty acid uptake and lipid synthesis (Torti et al. 1985). This fact, coupled with the observation that the serum of wasting animals can have elevated lipid levels, has led to the TNF-cachexia theory (Beutler and Cerami 1986).

When the ability of TNF-α to inhibit lipid anabolism in adipocytes is compared with that of other cytokines, we found that this property is not unique to TNF-α but is instead a general property of many cytokines (Patton et al. 1986). In addition to TNF-α and TNF-β, IFN-α, IFN-β, IFN-γ, and interleukin-1 appear to inhibit lipid anabolism (Keay and Grossberg 1980; Beutler and Cerami 1985; Patton et al. 1986). Table 4 shows the effect of a variety of cytokines on ^{3}H-labeled acetate uptake into lipid by 3T3 L1 fibroblasts and adipocytes. Both human TNF-α and TNF-β act across species boundaries to inhibit acetate uptake in mouse adipocytes. Murine IFN-γ exhibits 90% inhibition against mouse adipocytes, in contrast to human IFN-γ, which shows no effect. However, ^{3}H-labeled acetate uptake is inhibited by human IFN-α (hybrid IFN-α2/α1 [*BglII*]), which does exhibit cross-species antiviral activity. Uptake of ^{3}H-labeled acetate by undifferentiated adipocytes (3T3 L1 fibroblasts) is also inhibited by the cytokines, although not as markedly (35–40%) as in the differentiated cells. Furthermore, these same cytokines inhibit lipoprotein lipase activity (the enzyme responsible for removing lipid from the serum) and stimulate the release of fatty acids into the medium (Patton et al. 1986). Thus, the net effect of cytokine action on a fat cell is catabolic; fat uptake and synthesis are inhibited and fat mobilization is stimulated (a situation that arises during starvation).

The short-term in vivo catabolic effect of cytokines is to mobilize fat into the circulation for utilization by the immune system (Beutler and Cerami 1986). If the infection becomes chronic, wasting ensues. Although exudates from endotoxin-stimulated macrophages have been reported to cause dose-dependent wasting in mice when injected over a period of days (Cerami et al. 1985), we have been unable to induce wasting in rats with repeated injections of highly purified recombinant human TNF-α. In our experiment, the initial injections cause a loss of appetite and an increase in blood lipids that lasts for about 24 hours. However,

Table 4. Effect of Cytokines on ^{3}H-labeled Acetate Uptake in 3T3 L1 Adipocytes and Fibroblasts

Cell type	Cytokine	pmoles acetate uptake/hr/mg protein
3T3 L1 Murine Adipocytes	Control	53.6 ± 9.7[a]
	r-hTNF-α	4.6 ± 0.6[b]
	r-hTNF-α + r-hTNF-α antibody	57.6 ± 8.9
	r-hTNF-β	4.0 ± 0.4[b]
	r-mIFN-γ	5.3 ± 0.4[b]
	r-hIFN-γ	53.7 ± 7.7
	r-hIFN-α2/α1(*Bgl*II)	4.9 ± 0.4[b]
3T3 L1 Murine Fibroblasts	Control	8.0 ± 0.5
	r-hTNF-α	5.2 ± 1.0[c]
	r-hTNF-β	4.5 ± 0.6[d]
	r-mIFN-γ	5.2 ± 0.8[c]

Cells were treated with cytokines (~1.5 nM) for 24 hr and then given ^{3}H-labeled acetate for 1 hr.

[a]Mean ± S.D. ($n = 3$).

[b]$p < 0.0001$ relative to control value.

[c]$p < 0.005$ relative to control value.

[d]$p < 0.025$ relative to control value.

appetite returned and tolerance developed by the second day (J. Patton et al., unpubl.). Comparisons between these two experiments are difficult. The fact that rapid tolerance to TNF did develop is intriguing and we are investigating this further. Another question that merits further work is whether or not the cytotoxic effect of cytokines on certain cells is simply an expression of the same catabolic effects that are seen on fat cells.

The observation that a variety of cytokines can be catabolic suggests that release of individual cytokines in the animal may be separated in location and time. Perhaps during the multiple phases of host response to infection there is a succession of cytokines appearing and disappearing in the circulation or at the site of infection. Alternatively, different infections (e.g., viral, microbial, and parasitic) or different tissues subject to the same infection may induce a different set of cytokines. Thus, if energy mobilization is to occur in many types of infection or in many sites within the body, it is critical that several cytokines with overlapping biological function share this host defense activity.

TNF Receptor Binding Studies

Both TNF-α and TNF-β interact with cells via specific cell-surface receptors (Aggarwal et al. 1985a; Hass et al. 1985; Sugarman et al. 1985). The binding is both time- and temperature-dependent, reaching a maximum within 1 hour at 37°C. The binding requires 4–6 hours to plateau at 4°C. For both cytokines, a single class of high-affinity receptors with a K_d of approximately 10^{-10} M has been identified on a variety of tumor cell lines. In a few cases (e.g., 3T3 L1 adipocytes; Patton et al. 1986), both low- and high-affinity receptors for TNF-α were observed. Most of the tumor cells examined have 1000–5000 receptors per cell.

Whether full receptor occupancy is needed for the biological response of TNF-α or TNF-β is not yet clear. The concentration of TNF-β required for 50% killing of murine L929 cells is the same as that required to displace 50% of maximum binding, suggesting that full receptor occupancy is essential (Hass et al. 1985). However, the cytotoxic activity of TNF-α and TNF-β can be observed at severalfold lower concentrations with actinomycin-D-treated or mitomycin-C-treated cells. These metabolic inhibitors have no effect on the K_d of ligand binding, suggesting that under these conditions a very small fraction of the total receptors must be occupied for the cytotoxic response. The binding of ^{125}I-labeled TNF-α to cells can be effectively competed by unlabeled TNF-β, suggesting that both molecules are recognized by the same cell-surface receptor. This is probably a result of the structural similarity of TNF-α and TNF-β and explains their many common biological properties.

The receptor for TNF is probably a protein, since the binding of TNFs to cells can be abolished by pretreating the cells with proteolytic enzymes. After protease removal, binding of ligand can be completely restored in 24 hours (B.B. Aggarwal and T. Eessalu, unpubl.). The binding of some peptide hormones to cell surfaces can be abolished by gangliosides (Van Heyningen 1974). However, gangliosides were found to have no effect on the binding of TNFs to the TNF receptor.

The binding of TNFs to the TNF receptor is not directly correlated with effects on cell proliferation (Sugarman et al. 1985). Cell lines on which treatment with TNF-α or TNF-β had no effect on growth (e.g., T24 bladder carcinoma) bound these ligands with the same affinity as cell lines that were highly sensitive (Fig. 7). A cell line whose growth was inhibited by TNF (ME-180 cervical carcinoma) bound to the ligand with the same affinity as cells that were growth stimulated by TNF (WI38 lung fibroblasts). A similar number of receptors per cell were determined on all three cell types. The lack of correlation between binding and biological response of a given hormone has been observed previ-

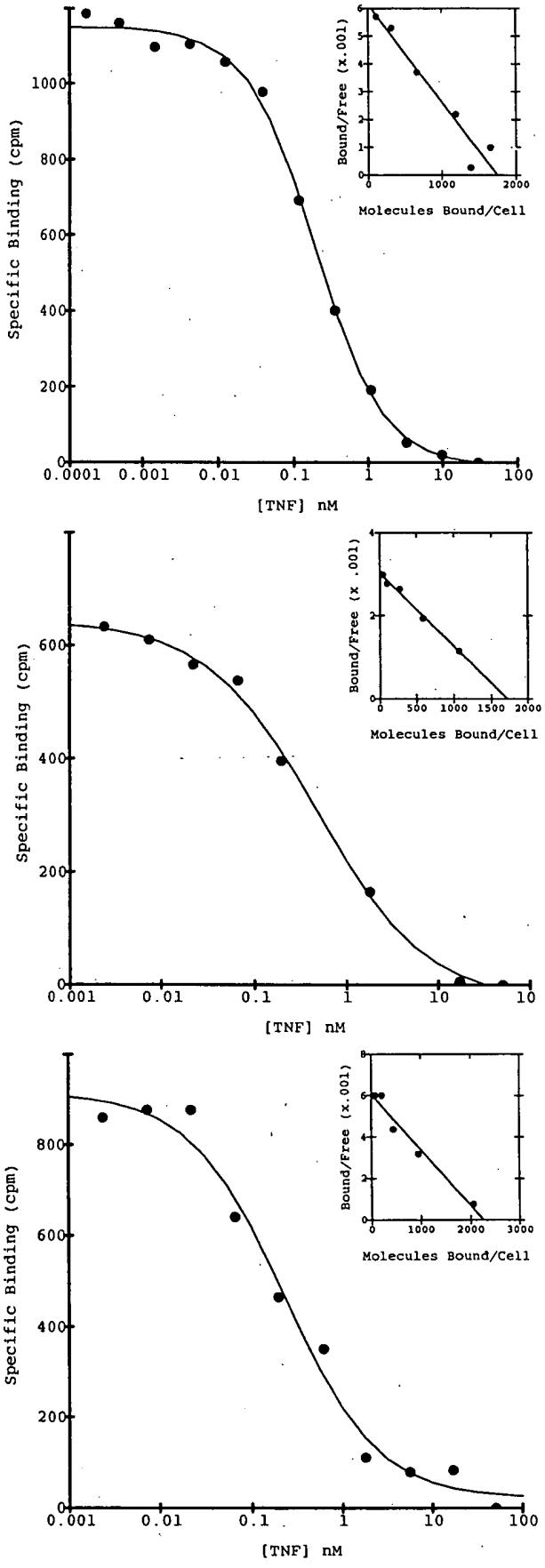

Figure 7. Competition curves of ^{125}I-labeled TNF-α with unlabeled TNF-α for binding to various cell lines. (*Top*) ME-180 cervical carcinoma; (*middle*) T24 bladder carcinoma; (*bottom*) WI38 lung fibroblast. (*Insets*) Scatchard analysis of the binding data. For experimental details, see Aggarwal et al. (1985a) and Hass et al. (1985).

ously for other proteins (Marchand-Brustel et al. 1985). This could be due to a cascade of events involved in the ultimate response of the cell to a given ligand. It is conceivable that nonresponsive cells may be defective for any one or more of these events.

We have reported that the cytotoxic activity of both TNF-α and TNF-β can be potentiated synergistically by IFN-γ (Lee et al. 1984; Sugarman et al. 1985). Although the mechanism of this synergy is not known, preexposure of cells to IFN-γ increases the total number of TNF receptors without affecting the affinity of the receptor-ligand interaction (Aggarwal et al. 1985a). A typical enhancement in TNF binding to B16 melanoma cells after exposure to IFN-γ is shown in Figure 8. Treatment of these cells for 16 hours with IFN-γ results in an approximately twofold increase in receptor number. Since this increase in TNF-α binding requires protein synthesis, it can be proposed that IFN-γ induces the de novo synthesis of TNF receptors. Whether the increase in receptor number is sufficient to explain synergistic action of TNF with IFN-γ is uncertain. Receptor induction may be just one part of the total mechanism of synergy. Since inhibitors of protein and RNA synthesis also potentiate the cytotoxic activity of TNFs, it is possible that IFN-γ also suppresses the synthesis of certain proteins that antagonize the actions of TNFs.

TNF-α and TNF-β display little species specificity. Radiolabeled human TNF-α and TNF-β bind to both human and murine cell lines, and this binding can be competed with both unlabeled murine and human TNFs. This is likely due to the close homology of human and murine TNFs (>80% amino acid sequence identity).

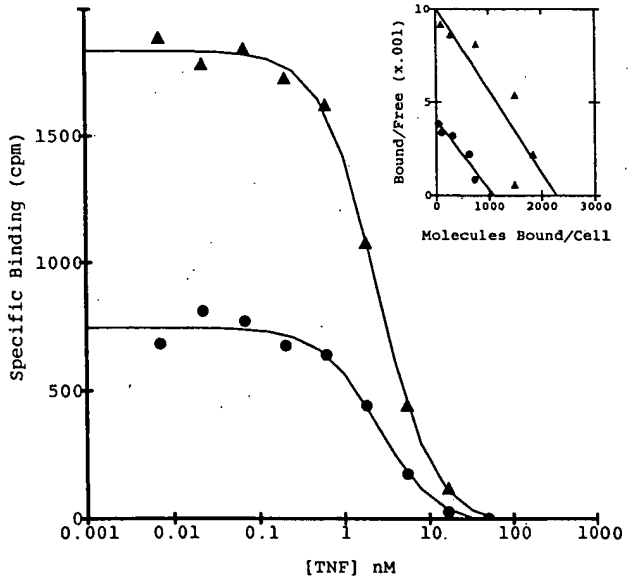

Figure 8. Induction of TNF-α receptors by IFN-γ on B16F10 melanoma cells. Competitive curves for untreated cells (●) and for cells treated overnight with IFN-γ (▲) are indicated. (*Inset*) Scatchard analysis of the binding data.

CONCLUSION

Our understanding of the TNF system has advanced tremendously over the past few years. The elucidation of the sequences of TNF-α and TNF-β by direct biochemical analysis and cDNA cloning has revealed that these cytokines are structurally related. Furthermore, the TNF genes are closely linked, their expression is tightly regulated, and the encoded gene products share many important biological properties. The biological activities of TNF-α and TNF-β are mediated through interaction with a common cell-surface receptor. The availability of cloned TNF genes and highly purified TNF preparations from recombinant *E. coli* should make it possible to address the many questions that remain unanswered concerning the regulation of TNF expression and the mechanisms of TNF action.

ACKNOWLEDGMENTS

We thank Drs. W. Wood and E. Chen for their help with bovine TNF-α and TNF-β sequence determination and Jeanne Arch for preparation of the manuscript.

REFERENCES

Aderka, D., H. Hoffmann, L. Toker, T. Hahn, and D. Wallach. 1986. Tumor necrosis factor induction by Sendai virus. *J. Immunol.* **136:** 2938.

Aggarwal, B.B., T.E. Eessalu, and P.E. Hass. 1985a. Characterization of receptors for human necrosis factor and their regulation by γ-interferon. *Nature* **318:** 665.

Aggarwal, B.B., B. Moffat, and R.N. Harkins. 1984. Human lymphotoxin. *J. Biol. Chem.* **259:** 686.

Aggarwal, B.B., W.J. Henzel, B. Moffat, W.J. Kohr, and R.N. Harkins. 1985b. Primary structure of human lymphotoxin derived from 1788 lymphoblastoid cell line. *J. Biol. Chem.* **260:** 2334.

Aggarwal, B.B., W.J. Kohr, P.E. Hass, B. Moffat, S.A. Spencer, W.J. Henzel, T.S. Bringman, G.E. Nedwin, D.V. Goeddel, and R.N. Harkins. 1985c. Human tumor necrosis factor: Production, purification and characterization. *J. Biol. Chem.* **260:** 2345.

Arya, S.K., F. Wong-Staal, and R.C. Gallo. 1984. Dexamethasone-mediated inhibition of human T cell growth factor and γ-interferon messenger RNA. *J. Immunol.* **133:** 273.

Babior, B.M. 1978. Oxygen-dependent microbial killing by phagocytes. *N. Engl. J. Med.* **298:** 659.

Bertolini, D.R., G.E. Nedwin, T.S. Bringman, D.D. Smith, and G.R. Mundy. 1986. Stimulation of bone resorption and inhibition of bone formation *in vitro* by human tumor necrosis factors. *Nature* **319:** 516.

Beutler, B.A. and A. Cerami. 1985. Recombinant interleukin 1 suppresses lipoprotein lipase activity in 3T3 L1 cells. *J. Immunol.* **135:** 3969.

———. 1986. Cachectin and tumour necrosis factor as two sides of the same biological coin. *Nature* **320:** 584.

Beutler, B., N. Krochin, I.W. Milsark, C. Luedke, and A. Cerami. 1986. Control of cachectin (tumor necrosis factor) synthesis: Mechanisms of endotoxin resistance. *Science* **232:** 977.

Carswell, E.A., L.J. Old, R.L. Cassel, S. Green, N. Fiore, and B. Williamson. 1975. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci.* **72:** 3666.

Cerami, A., Y. Ikeda, N. Le Trang, P.J. Hotez, and B. Beutler. 1985. Weight loss associated with an endotoxin-induced mediator from peritoneal macrophages: The role of cachectin (tumor necrosis factor). *Immunol. Lett.* **11:** 173.

Claman, H.N. 1972. Corticosteroids and lymphoid cells. *N. Engl. J. Med.* **287:** 388.

Culpepper, J.A. and F. Lee. 1985. Regulation of IL-3 expression by glucocorticoids in cloned murine T lymphocytes. *J. Immunol.* **135:** 3191.

Goodwin, J.S. and D.R. Webb. 1980. Regulation of immune response by prostaglandin. *Clin. Immunol. Immunopathol.* **15:** 106.

Granger, G.A. and W.P. Kolb. 1968. Lymphocyte *in vitro* cytotoxicity: Mechanisms of immune and non-immune small lymphocyte mediated target L-cell destruction. *J. Immunol.* **101:** 111.

Gray, P.W. 1986. Molecular characterization of human lymphotoxin. In *Gene cloning in lymphokine research* (ed. D. Webb and D.V. Goeddel). Academic Press, New York. (In press.)

Gray, P.W., B.B. Aggarwal, C.V. Benton, T.S. Bringman, W.J. Henzel, J.A. Jarrett, D.W. Leung, B. Moffatt, P. Ng, L.P. Svedersky, M.A. Palladino, and G.E. Nedwin. 1984. Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumor necrosis activity. *Nature* **312:** 721.

Harlan, J.M., P.D. Killen, L.A. Harker, G.E. Stricker, and D.G. Wright. 1981. Neutrophil mediated endothelial injury *in vitro*: Mechanisms of cell detachment. *J. Clin. Invest.* **68:** 1394.

Hass, P.E., A. Hotchkiss, M. Mohler, and B.B. Aggarwal. 1985. Characterization of specific high affinity receptors for human tumor necrosis factor on mouse fibroblasts. *J. Biol. Chem.* **260:** 12214.

Itoh, H. and R.B. Wallace. 1985. A novel human physiologically active polypeptide. *European Patent Application* No. 84105149.3.

Keay, S. and S.E. Grossberg. 1980. Interferon inhibits the conversion of 3T3 L1 fibroblasts into adipocytes. *Proc. Natl. Acad. Sci.* **77:** 4099.

Kohase, M., D. Henriksen-DeStefano, L.T. May, J. Vilcek, and P.B. Sehgal. 1986. Induction of β_2-interferon by tumor necrosis factor: A homeostatic mechanism in the control of cell proliferation. *Cell* **45:** 659.

Kramer, S.M. and M.E. Carver. 1986. Serum-free *in vitro* bioassay for the detection of tumor necrosis factor. *J. Immunol. Methods* (in press).

Kull, F.C., S. Jacobs, and P. Cuatrecasas. 1985. Cellular receptor for ^{125}I-labeled tumor necrosis factor: Specific binding, affinity labeling, and relationship to sensitivity. *Proc. Natl. Acad. Sci.* **92:** 5756.

Lee, S.H., B.B. Aggarwal, E. Rinderknecht, F. Assisi, and H. Chiu. 1984. The synergistic and anti-proliferative effect of γ-interferon and human lymphotoxin. *J. Immunol.* **133:** 1083.

Marchand-Brustel, Y.L., T. Gremeaux, P. Ballotti, and E. Van Obberghen. 1985. Insulin receptor tyrosine kinase is defective in skeletal muscle of insulin resistant obese mice. *Nature* **315:** 676.

Nawroth, P.P. and D.M. Stern. 1986. Modulation of endothelial cell homeostatic properties by tumor necrosis factor. *J. Exp. Med.* **163:** 740.

Nedwin, G.E., S.L. Naylor, A.Y. Sakaguchi, D. Smith, J. Jarrett-Nedwin, D. Pennica, D.V. Goeddel, and P.W. Gray. 1985. Human lymphotoxin and tumor necrosis factor genes: Structure, homology and chromosomal localization. *Nucleic Acids Res.* **13:** 6361.

Old, L.J. 1985. Tumor necrosis factor (TNF). *Science* **230:** 630.

Patton, J.S., H.M. Shepard, H. Wilking, G.D. Lewis, B.B. Aggarwal, T.E. Eessalu, L.A. Gavin, and C. Grunfeld. 1986. Interferons and tumor necrosis factors have similar catabolic effects on 3T3 L1 cells. *Proc. Natl. Acad. Sci.* (in press).

Pennica, D. and D.V. Goeddel. 1986. Cloning and characterization of the genes for human and murine tumor necrosis

factors. In *Gene cloning in lymphokine research* (ed. D. Webb and D.V. Goeddel). Academic Press, New York. (In press.)

Pennica, D., M.R. Shalaby, and M.A. Palladino. 1986. Tumor necrosis factors alpha and beta. In *Recombinant lymphokines and their receptors* (ed. S. Gillis). Marcel Dekker, New York. (In press.)

Pennica, D., J.S. Hayflick, T.S. Bringman, M.A. Palladino, and D.V. Goeddel. 1985. Cloning and expression in *Escherichia coli* of the cDNA for murine tumor necrosis factor. *Proc. Natl. Acad. Sci.* **82:** 6060.

Pennica, D., G.E. Nedwin, J.S. Hayflick, P.H. Seeburg, R. Derynck, M.A. Palladino, W.J. Kohr, B.B. Aggarwal, and D.V. Goeddel. 1984. Human tumor necrosis factor: Precursor structure, expression, and homology to lymphotoxin. *Nature* **312:** 724.

Rager-Zisman, B. and T.C. Merigan. 1973. A useful quantitative semimicromethod for viral plaque assay (37202). *Proc. Soc. Exp. Biol. Med.* **142:** 1174.

Roberts, A.B., M.. Anzano, L.M. Wakefield, N.S. Roche, D.F. Stern, and M.B. Sporn. 1985. Type β transforming growth factor: A bifunctional regulator of cellular growth. *Proc. Natl. Acad. Sci.* **82:** 119.

Ruddle, N.H. and B.H. Waksman. 1968. Cytotoxicity mediated by soluble antigens and lymphocytes in delayed hypersensitivity. *Science* **157:** 1060.

Ruddle, N.H., M.B. Powell, and B.S. Conta. 1983. Lymphotoxin, a biologically relevant model lymphokine. *Lymphokine Res.* **2:** 23.

Ruggiero, V., J. Tavernier, W. Fiers, and C. Baglioni. 1986. Induction of the synthesis of tumor necrosis factor receptors by interferon-γ. *J. Immunol.* **136:** 2445.

Shalaby, M.R., B.B. Aggarwal, E. Rinderknecht, L.P. Svedersky, B.S. Finkle, and M.A. Palladino. 1985. Activation of human polymorphonuclear neutrophil functions by gamma interferon and tumor necrosis factors. *J. Immunol.* **135:** 2069.

Shen, T. and C.A. Winter. 1977. Chemical and biological studies on indomethacin, sulindac and their analogs. *Adv. Drug. Res.* **12:** 89.

Stewart, W.E., II. 1981. *The interferon system*. Springer-Verlag, Vienna.

Stone-Wolff, D.S., Y.K. Yip, H.C. Kelker, J. Le, D. Henriksen-DeStefano, B.Y. Rubin, E. Rinderknecht, B.B. Aggarwal, and J. Vilcek. 1984. Interrelationship of human interferon-gamma with lymphotoxin and monocyte cytotoxin. *J. Exp. Med.* **159:** 828.

Sugarman, B.J., B.B. Aggarwal, P.E. Hass, I.S. Figari, M.A. Palladino, and H.M. Shepard. 1985. Recombinant human tumor necrosis factor-α: Effects on proliferation of normal and transformed cells *in vitro*. *Science* **230:** 943.

Tanaka, K., K.J. Isselbacher, G. Khoury, and G. Jay. 1985. Reversal of oncogensis by the expression of a major histocompatibility complex class I gene. *Science* **228:** 26.

Thomas, P.S. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci.* **77:** 5201.

Tiku, K., M.L. Tiku, and J.L. Skosey. 1986. Interleukin 1 production by human polymorphonuclear neutrophils. *J. Immunol.* **136:** 3677.

Torti, F.M., B. Dieckmann, B. Beutler, A. Cerami, and G.M. Ringold. 1985. A macrophage factor inhibits adipocyte gene expression: An *in vitro* model of cachexia. *Science* **229:** 867.

Tsujimoto, M., Y.K. Yip, and J. Vilcek. 1986. Inteferon-γ enhances expression of cellular receptors for tumor necrosis factor. *J. Immunol.* **136:** 2441.

Tucker, R.F., G.D. Shipley, H.L. Moses, and R.W. Holley. 1984. Growth inhibitor from BSC-1 cells closely related to platlet type β transforming growth factor. *Science* **226:** 705.

Urban, J.L., H.M. Shepard, J.L. Rothstein, B.J. Sugarman, and H. Schreiber. 1986. Tumor necrosis factor: A potent effector molecule for tumor cell killing by activated macrophages. *Proc. Natl. Acad. Sci.* **83:** 5233.

Van Heyningen, W.E. 1974. Gangliosides as membrane receptors for tetanus toxin, cholera toxin and seratonin. *Nature* **249:** 415.

Vilcek, J., V.J. Palombella, D. Henricksen-DeStefano, C. Swenson, R. Feinman, M. Hirai, and M. Tsujimoto. 1986. Fibroblast growth enhancing activity of tumor necrosis factor and its relationship to other polypeptide growth factors. *J. Exp. Med.* **163:** 632.

Vischer, T.L. 1972. Effect of hydrocortisone on the reactivity of thymus and spleen cells of mice to *in vitro* stimulation. *Immunology* **23:** 77.

Williams, T.W. and J.A. Bellanti. 1983. *In vitro* synergism between human interferons and human lymphotoxins: Enhancement of lymphotoxin-induced target cell killing. *J. Immunol.* **130:** 518.

Williamson, B.D., E.A. Carswell, B.Y. Rubin, J.S. Prendergast, and L. Old. 1983. Human tumor necrosis factor produced by human B-cell lines: Synergistic cytotoxic interaction with human interferon. *Proc. Natl. Acad. Sci.* **80:** 5397.

Wong, G.H.W., I. Clark-Lewis, J.A. Hamilton, and J.W. Schrader. 1984. P cell stimulating factor and glucocorticoids oppose the action of interferon-γ in inducing Ia antigens on T-dependent mast cells (P cells). *J. Immunol.* **133:** 2043.